临床研究

Survivin和PI3K、AKT在寻常型银屑病皮损角质形成细胞中的表达及其相关性

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摘要:目的 探讨凋亡抑制蛋白Survivin和PI3K、AKT在寻常型银屑病(PV)进行期斑块状皮损角质形成细胞(KCs)中的表达水平及作用。**方法** 临床上收集22例PV患者进行期斑块状皮损以及18例正常皮肤组织。采用免疫组织化学、Western blot和real-time quantitative PCR等方法检测22例PV患者进行期斑块状皮损和18例正常皮肤组织中KCs的Survivin、PI3K和AKT表达,并分析它们在PV皮损KCs中的相关性。使用小干扰RNA(siRNA)在HaCaT细胞中沉默AKT,并通过Western blot实验检测Survivin的表达水平。**结果** PV皮损KCs中Survivin、PI3K和AKT的蛋白表达水平均高于正常皮肤组织。PV皮损KCs中Survivin和PI3K mRNA表达成正相关(r=0.4510;P=0.0351);PV皮损KCs中Survivin和AKT mRNA表达成正相关(r=0.4423;P=0.0393);在HaCaT细胞中,沉默AKT后可引起Survivin的表达下调。**结论** 凋亡抑制蛋白Survivin与PI3K/AKT信号通路可能参与了PV的发生与发展。

关键词: Survivin;PI3K;AKT;寻常型银屑病

Expressions of survivin, PI3K and AKT in keratinocytes in skin lesions and their pathogenic role in psoriasis vulgaris

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Abstract: Objective To explore the role of survivin and PI3K/AKT pathway in the pathogenesis of psoriasis vulgaris (PV). **Methods** Plaque-like lesions collected from 22 patients with PV in progressive stage and 18 normal control skin specimens were examined using immunohistochemical staining, Western blotting and real-time quantitative PCR for expressions of survivin, PI3K and AKT in the keratinocytes, and their correlation was analyzed. A small interfering RNA (siRNA) was used to knock down AKT in cultured HaCaT cells, and Western blotting was used to detect the changes in the expression of survivin. **Results** Compared with normal skin, PV lesions showed obviously up-regulated expressions of survivin, PI3K and AKT in the keratinocytes. Survivin expression was positively correlated with PI3K (r=0.4510, P=0.0351) and AKT (r=0.4423, P=0.0393) in the keratinocytes in PV lesions. In cultured HaCaT cells, siRNA-mediated knockdown of AKT caused down-regulation of survivin expression. **Conclusion** Survivin and PI3K/AKT signaling pathway may participate in the occurrence and progression of PV.

Keywords: Survivin; PI3K; AKT; psoriasis vulgaris

银屑病是临床常见的一种慢性复发性的炎症性皮肤病,超过90%的银屑病患者为寻常型银屑病(PV),其组织病理表现主要为表皮角质增厚、真皮乳头层血管增生和炎症细胞浸润[1-3]。然而,银屑病的病因及发病机制尚不完全清楚。存活素(Survivin)作为一种凋亡蛋白抑制因子(IAP),广泛表达于各种肿瘤组织中,如乳腺癌、口腔癌、肺癌和肝癌等[4-7]。然而,目前Survivin在银屑病中的表达水平仍存在争议。磷脂酰肌醇3-激酶(PI3K)/蛋白激酶B(AKT)信号通路是肿瘤研究的热门信号通路,它参与了肿瘤的增殖、转移、耐药以及凋亡抑制等过程[8]。目前研究发现其与银屑病的发生发展可能相关[9-10]。为进一步探索Survivin、PI3K、AKT在银屑病发病机制中的作用,本研究在基因、蛋白、细胞水平并

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应用小干扰RNA(siRNA)策略,检测人银屑病皮损角质形成细胞(KCs)中Survivin、PI3K和AKT蛋白和mRNA的表达水平并探讨其相关性和意义。

1 材料和方法

1.1 临床资料

寻常型银屑病(PV)患者22例,均来自西安交通大学第二附属医院皮肤科门诊患者,患者均具有典型的临床症状及病理特征,符合PV进行期的诊断标准,其中男性12例,女性10例,年龄24~60岁,平均41.68岁;病程3月~22年,平均7.63年。以同期我院皮肤外科18例美容整形患者切除的正常皮肤组织作为对照组,其中男性10例,女性8例,年龄15~51岁,平均39.81岁。所有银屑病患者取材前3月未服用或外用糖皮质激素、免疫抑制剂及维A酸类药物,取材前2周内未使用任何药物治疗,且不伴有其他皮肤及系统疾病。所有标本取材均按常规皮肤活检手术进行。银屑病患者组与健康对照组在年龄、性别等方面比较差异无统计学意义(P>0.05)。

1.2 免疫组织化学

采用EnVision二步法:65 ℃烤片,二甲苯脱蜡,酒精梯度水化,高压锅抗原修复5 min,阻断内源性过氧化物酶10 min,一抗(Survivin、PI3K、AKT均购买于北京博奥森生物公司)(浓度参考抗体说明书)孵育4 ℃过夜,二抗常温孵育30 min,DAB显色,苏木素复染,盐酸分化和氨水反蓝,酒精梯度脱水,二甲苯透明和中性树胶封片,最后再显微镜下观察和拍片。免疫组织化学染色评分:根据着色强度(无、弱、中等、强)分别评0、1、2、3分;根据阳性细胞百分比[(0~25)%、(25~50)%、(50~75)%、(75~100)%]分别评1、2、3、4分,总分为着色强度评分和阳性细胞百分比评分的乘积。

1.3 细胞培养

将HaCaT细胞株培养于含10%胎牛血清的1640培养基(Gibco)中,并将细胞置于37 ℃、5% CO_2的培养箱中进行培养。

1.4 siRNA转染

AKT siRNA(吉玛生物),siAKT序列为:正义链5'-UUGUAGCCAAUGAAGGUGCCA-3',反义链5'-GCACCUUCAUUGGCUACAATT-3',转染试剂X-treme-GENEsiRNA transfection reagents购自Roche公司。将1.5×10^5 HaCaT细胞种植于6孔细胞培养板。24 h后采用X-tremeGENE siRNA transfection reagents将2 μg/孔siRNA转染到细胞内,继续培养48 h后提取细胞总蛋白。

1.5 Western blot

常规提取组织和细胞总蛋白,使用10%的SDS-PAGE凝胶进行电泳分离蛋白,电泳后采用湿转的方法将蛋白转移至PVDF膜上,5%脱脂牛奶常温封闭1 h,一抗(Survivin、PI3K、AKT和GAPDH均购买于北京博奥森生物公司)孵育4 ℃过夜,TBST洗涤3次,5 min/次,HRP标记的二抗(CST)常温孵育1 h,TBST洗涤3次,5 min/次,在暗室中将PVDF膜蛋白面浸入HRP-ECL发光液中,并使用ChemiDoc XRS成像系统显影。使用GAPDH作为内参。

1.6 Real-time PCR

使用Trizol试剂提取组织和细胞中的总RNA。使用PrimeScript™ RT Master Mix (Perfect Real-time)反转录试剂盒对所提取的RNA进行反转录。将反应物混匀后,放入MyCycler™普通PCR仪中进行反转录。当成功反转录为cDNA后,使用SYBR® Premix Ex Taq™ II (TliRNaseH Plus)试剂盒进行Real-time quantitative PCR实验。其中引物序列为:Survivin,上游引物5'-CACCCCGGAGCGGATGG-3',下游引物5'- GTCATCTGGCTCCCAGCCTTC-3';AKT,上游引物5'-AGCGACGTGGCTATTGTGAAG-3',下游引物5'-GCCATCATTCTTGAGGAGGAAGT-3';PI3K,上游引物5'-TATTTGGACTTTGCGACAAGACT-3',下游引物5'-TCGAACGTACTGGTCTGGATAG-3';GAPDH,上游引物5'-GGAGCGAGATCCCTCCAAAAT-3',下游引物5'-GAACCTGGAAGAGTCCGAAGTA-3'。其中GAPDH作为内参。

1.7 统计学方法

使用Graphpad Prism 5.0版本软件分析两组之间的差异(two-tailed student's t检验),采用Pearson correlation分析Survivin与PI3K、AKT表达的相关性。当P<0.05认为差异有统计学意义。

2 结果

2.1 免疫组织化学和Western blot检测

与正常皮肤相比,Survivin、PI3K和AKT蛋白在PV皮损组织KCs中表达上调(图1)。

2.2 Real-time quantitative PCR检测及相关性分析

Survivin和PI3K mRNA的表达呈正相关(r=0.4510;P=0.0351);Survivin和AKT mRNA表达呈正相关(r=0.4423;P=0.0393,图2)。

2.3 siRNA干扰技术

在HaCaT细胞中,沉默AKT可以下调Survivin的表达(图3)。

3 讨论

Survivin作为凋亡蛋白抑制因子(IAP)家族成员中抗凋亡作用最强的蛋白之一,目前广泛的在各种肿瘤组织中表达,如乳腺癌、口腔癌、肺癌和肝癌等[4-7]。大量研

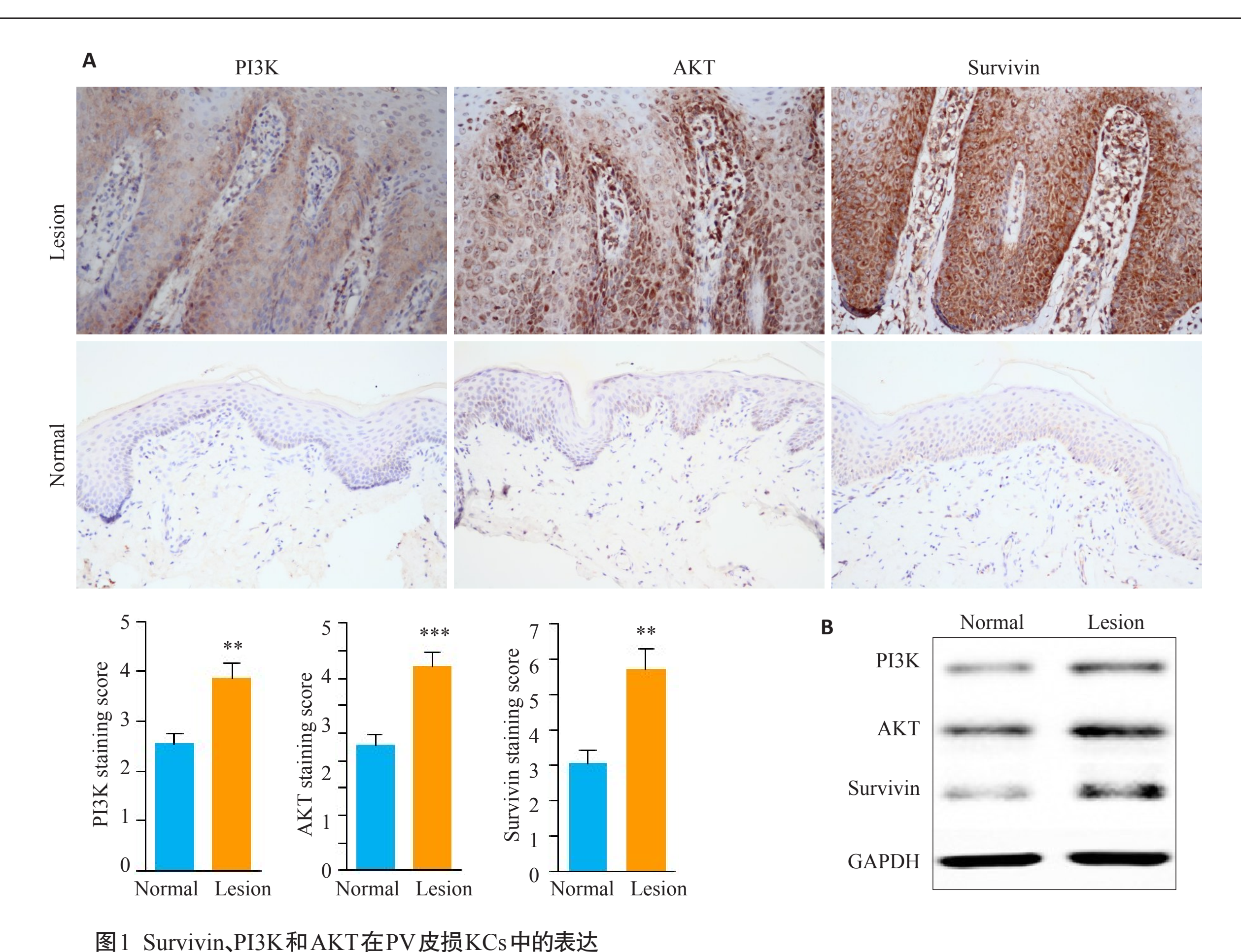




图1 Survivin、PI3K和AKT在PV皮损KCs中的表达

Fig.1 Expression of survivin, PI3K and AKT in the keratinocytes inPV lesions. *A*: Immunohistochemical staining of survivin, PI3K and AKT in the keratinocytes in normal skin and PV lesions (Original magnification: ×400); *B*: Western blot analysis of survivin, PI3K and AKT in the keratinocytes in normal skin and PV lesions. ***P*<0.01, ****P*<0.001 *vs* normal skin.

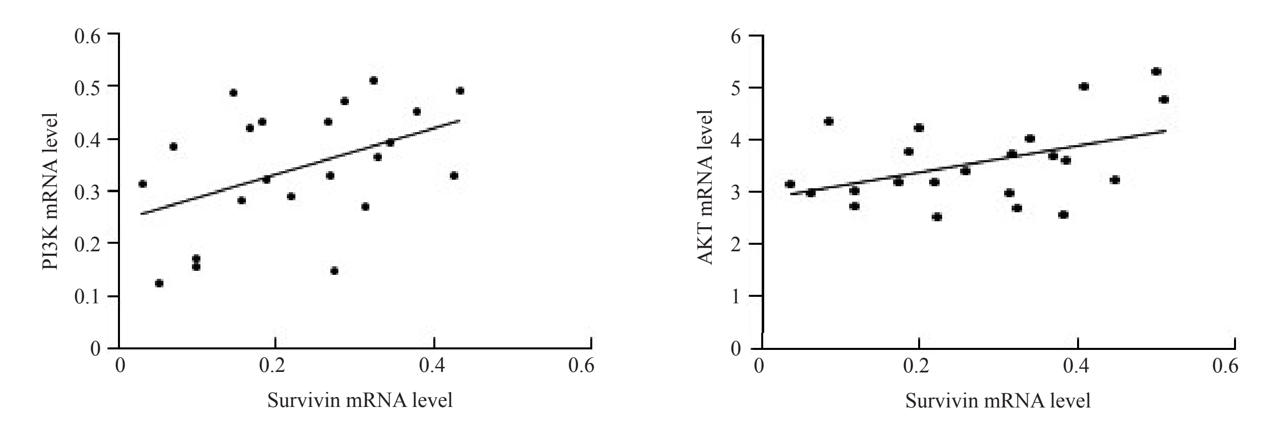


图2 PV皮损KCs中Survivin、PI3K和AKT的相关性分析

Fig.2 Correlation analysis of survivin, PI3K and AKT in the keratinocytes in PV lesions. Left: Correlation analysis between survivin and PI3K mRNA; Right: Correlation analysis between survivin and AKT mRNA.

究发现Survivin在肿瘤中高表达且可以抑制肿瘤细胞的凋亡,从而促进肿瘤的发生、增殖和对放射治疗以及化学治疗的抵抗[6, 11-13]。PV是一种慢性炎症性、增殖性皮肤病,角质形成细胞增殖过度和凋亡的缺失不足是PV发生发展的机制之一[1-2]。目前有关Survivin在PV皮损中的表达水平尚有争议。Bowen等[14]发现最先报道Survivin在皮肤肿瘤和角质细胞增生皮损中表达增高,而在正常皮肤中表达较低。Simonetti等[15]报道Survivin和血管内皮生成因子(VEGF)在PV皮损中均表达上调。同样,Wang等[16]发现Survivin不仅在PV皮损中表达上调,且与抗菌肽β-defensin-3表达呈正相关。然而,Gunduz K等[17]报道PV皮损中survivin蛋白

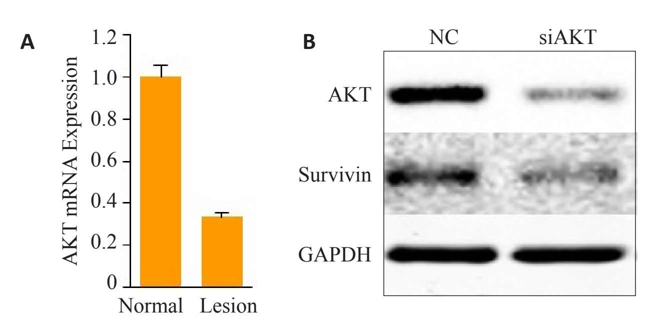


图3 PV皮损KCs中PI3K/AKT信号通路和Survivin的相关性

Fig.3 Correlation between PI3K/AKT signaling pathway and survivin in the keratinocytes in PV lesions. *A*: Real-time quantitative PCR of AKT in HaCaT NC and AKT-deficient sublines; *B*: Western blot analysis of AKT and survivin in HaCaT NC and AKT deficient sublines.

表达水平与正常皮肤中的表达水平没有差异。为了进一步明确survivin在PV皮损中的表达情况,我们在蛋白和mRNA水平上进行探究,发现survivin mRNA和蛋白水平在PV皮损中的表达水平均高于正常皮肤。

PI3K/AKT信号通路在细胞生长、增殖等功能中起重要的作用[18-19]。AKT是PI3K下游的靶蛋白,可以通过磷酸化其下游mTOR、Bad等蛋白,从而调控细胞的增殖和凋亡[20, 23]。大量研究证明,PI3K/AKT信号通路在肿瘤增殖和凋亡异常中起到重要作用[24-25]。在PV发生发展过程中,角质形成细胞的增殖和凋亡失衡起着重要的作用。Chamcheu等[9-10]发现在Imiquimod诱导的鼠PV样皮炎模型中,PI3K/AKT信号活性是增强的,进一步发现抑制PI3K/AKT信号通路可以缓解Imiquimod诱导的鼠PV样皮损。Jeon等[26]报道抑制PI3K/AKT信号通路可以缓解皮肤的炎症反应。然而,PI3K和AKT在人PV皮损中的表达尚不清楚。在我们的研究中发现,与正常皮肤相比,PV皮损KCs中的PI3K和AKT的表达均上调,提示PI3K/AKT信号通路在PV发生发展中具有重要的作用。

PI3K/AKT信号通路参与了肿瘤细胞的增殖和凋亡异常。然而,在PV中,尚不清楚PI3K/AKT信号通路是否参与了促进角质形成细胞(KCs)增殖、抑制KCs凋亡的过程。本研究检测了PV皮损中Survivin、PI3K和AKTmRNA的表达,并对Survivin和PI3K以及Survivin和AKT进行了相关性分析。我们发现Survivin和PI3K的表达呈正相关;Survivin和AKT的表达也呈正相关,表明PI3K/AKT信号通路可能通过Survivin参与了PV皮损中KCs的凋亡受抑。另外,在体外细胞实验中,我们发现沉默HaCaT细胞AKT可以下调其Survivin蛋白的表达,提示KCs中AKT可以正向调控其Survivin的表达。

目前普遍认为,IL-23/Th17(白介素-23/辅助性T细胞17)轴的失调引起并加剧PV患者皮损中慢性炎症反应[27]。随着对银屑病发病机制研究的深入,人们发现各种细胞因子及炎性细胞、炎性介质在银屑病的发病中都不是单一发挥作用的,而是相互制约、相互影响,构成一个复杂的网络。在银屑病发病过程中,炎症、增殖与凋亡密切相关。

总之,我们的研究初步发现Survivin与PI3K、AKT在PV皮损KCs中蛋白表达均升高,并在mRNA表达水平呈正相关,沉默KCsAKT可以下调其Survivin蛋白的表达,表明PI3K/AKT信号通路可能通过凋亡抑制蛋白Survivin参与了PV的发生与发展,提示Survivin与PI3K/AKT信号通路可为银屑病治疗潜在的新靶点,可能为银屑病的治疗提供新的思路。

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